Thermal Stability: Cx26 is least stable in UDM and most stable in DDM+CHS

Cooperative unfolding revealed by fluorescence

In an effort to optimize the protein detergent complex for 3D crystallization, we measured the thermal stability of hexameric Cx26 in several detergents. Thermal stability is measured using a simple dye accessibility assay in which a small amount of fluorophore dye intercalator enters the channel under large increase in quantity. Cx26 displays a denaturation profile in dodecylmaltoside suggesting a simple 2-state cooperative transition. Additional conditions are currently being tested.

We are encouraged that the expression levels for several Cx26 mutants are sufficient for structural studies. The micelle expression and stability assay will be valuable for optimization of mutant, detergent, buffer and additive conditions to accelerate crystallization trials.

Membrane topology of connexin. Members of the connexin gene family contain four transmembrane domains (M1-M4), two extracellular loops (EL1 and EL2), a cytoplasmic loop (IL) between M3 and M4, and N and C-termini. A hexameric hemichannel, also called a connexon, docks end-to-end with a second hexamer to form the intercellular channel. It is likely that modifications of wildtype, full length Cx26 will be required to achieve a high resolution crystal structure. The full length construct was used as a starting point for an extensive mutagenesis and expression screen.

Results:
• There is robust expression of wildtype Cx26 in SF9 cells, and some truncation mutants displayed even higher levels of expression.
• Truncations at the N- or C-terminus, and to a lesser extent the EL1 loop, resulted in no recombinant virus or a reduction of expression.
• Truncation of the cytoplasmic M2-M3 loop (IL) and EL2 were surprisingly well tolerated.
• Selected truncations involving both termini and IL have been expressed and purified at the 1 L scale, yielding milligram quantities of purified protein.
• The relative expression levels observed in the small scale analysis have translated well to scale-up to one liter.

Expression screening: Truncations in the M2-M3 cystolic loop (IL) and EL2 are well tolerated


Thermal Stability of Human Connexin 26

Kent A. Baker1,2, Sri Dhyna1, Ellen Chien1, Yuanzi Hua2, Mark Yeager1,2,3,4, Raymond C. Stevens1

1Department of Molecular Biology, The Scripps Research Institute, La Jolla, CA; 2Department of Cell Biology, The Scripps Research Institute, La Jolla, CA; 3Division of Cardiomyopathies and Cardiomyocardial Diseases, Scripps Clinic, La Jolla, CA; 4Dept. of Molecular Physiology and Biological Physics, University of Virginia Health System, Charlottesville, VA.

Supported by NIH Roadmap grant P50 GM073197 (Pl: RCS) and NIH RO1 HL048908 (MY).